EXPERIMENTAL LOCALIZATION OF NEW AXES IN CORYMORPHA WITHOUT OBLITERATION OF THE ORIGINAL POLARITY.

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The various lines of evidence demonstrating the existence of physiological gradients in *Corymorpha* have been considered in earlier papers (Child and Hyman, '26, Child, '26*a*, '26*b*). In the last of these papers it was shown that the differential resulting from contact of one end of a stem piece with the bottom and free exposure of the other may determine the one as basal, the other as apical, irrespective of the original polarity. In accordance with this fact it was shown that in pieces undergoing reconstitution on the bottom the bipolar frequency is lower and the unipolar frequency higher than in those supported on loose cotton near the surface of the water so that the ends are more nearly equally exposed. In other papers it was shown that pieces after subjection to various inhibiting agents may develop new polarities and symmetries quite independent of the original axes and of the cut ends (Child, '27*a, b*). Apparently the inhibiting agents decrease or obliterate the original polarity and symmetry and the localizing influence of the cut ends and under these conditions the differential of position becomes more effective in localizing apical ends on the free surface and basal ends on the surface in contact or near the bottom.

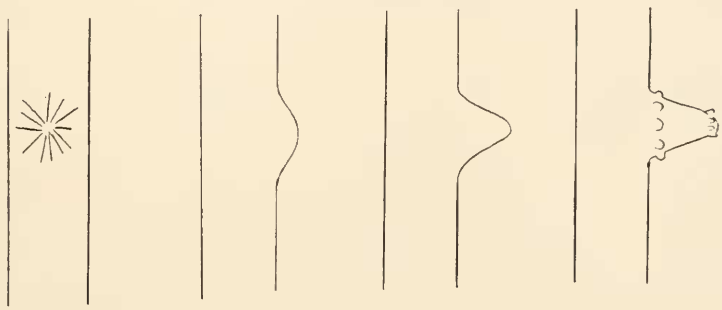
The present paper is concerned with some further experiments on the determination of new polarities. In these experiments the new axes are localized as centers of high metabolic activity and growth without obliterating the original polarity.

Experimental.

No indication of development of new hydranths by budding has been observed in *Corymorpha*. Among thousands of individuals collected, only a single case of more than one hydranth on one stem has been observed. In this case the stem was single over most of its length, but divided into two distally and each of these bore a hydranth. The two hydranths were equal in size and it was not possible to distinguish one as terminal, the other as lateral. Another individual collected possessed a lateral stem outgrowth some ten mm. in length but without hydranth or base at its tip. A third individual possessed two manubria. These are the only cases of axial multiplication found thus far in the collected material. Considering the high frequency of such multiplication under experimental conditions (Child, '27 *a, b*) it seems remarkable that it does not occur more frequently in nature.

A simple lateral cut with smooth edges, extending a third or even half way through the stem closes within an hour or two under normal conditions and no new apical end or other outgrowth results from it. An earlier experiment on pieces of the actinian, *Harenactis,* suggested that a modification of the procedure employed in that case might determine a new polarity and symmetry in lateral stem regions of *Corymorpha.* In the case of *Harenactis* it was found that when mesenteries and muscles were much injured or in large part removed the shorter transverse pieces contracted in such a way as to bring distal and proximal cut edges of the body wall together and union took place between these edges about the whole circumference, giving rise to 'rings' (Child, '09*b*). It was found further that in places where the union between the cut edges was smooth and without much new tissue no outgrowths developed along the line of union, while in places where more new tissue developed groups of tentacles appeared. This result led to the further experiment of mutilating opposite regions of the two cut edges by means of numerous small cuts close together and vertical to the edge. When these two mutilated regions came together they could not unite smoothly and extensive growth of new tissue took place before healing was complete. From this new tissue there gradually developed in some cases a new normal individual (Child, '10, Figs. 5, 6). The new apical region appeared only after complete closure of the wound by new tissue. This new tissue gradually bulged outward because of the internal water pressure, continued to grow and finally developed as a new polar axis. The radial symmetry accompanying this new polarity seemed to be primarily merely an expression of the likeness of all radii in a plane vertical to the polar axis.

In the hope that it might be possible to determine a new polarity from the lateral stem region of *Corymorpha* in a manner somewhat similar to that employed in *Harenactis* the stems were cut as follows: with small, fine-pointed scissors cuts one to two mm. in length, radiating from a center, were made as indicated in Fig. 1, the purpose of the operation being merely to localize a region



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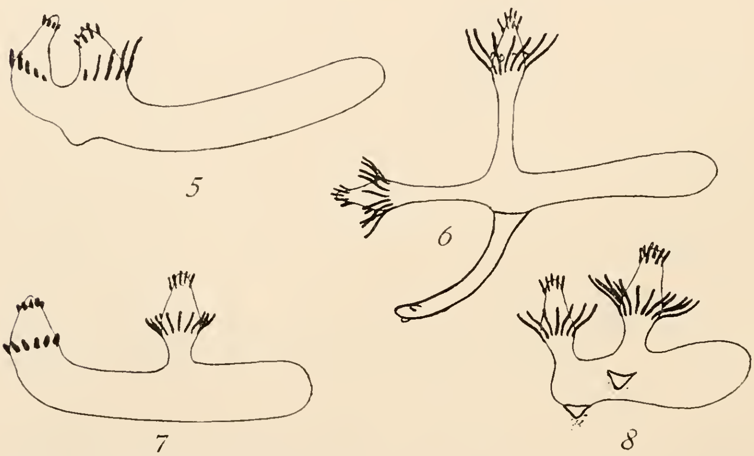
FIGS. 1–4. Development of hydranth from lateral region of growth determined by radiating cuts as shown in Fig. 1.

of active cells. In all cases the original hydranth was removed in order that its dominance might not interfere with the development of a lateral hydranth and in some of the earlier series the new hydranths which developed at one or both ends of the piece were also removed in early stages for the same reason, but this was found to be unnecessary. Closure of this wound was slower than in case of a simple cut, but was usually complete in twelve to twenty-four hours. In the successful operations the region began to bulge soon after closure (Fig. 2) and soon became a definite rounded outgrowth which underwent elongation (Fig. 3) and after two or three days attained the form of a hydranth with early stages of tentacles (Fig. 4).

Figures 5–18 show characteristic results of this operation. In all figures the region of stem covered with perisarc is indicated by heavier outline than other regions and the perisarcal accumulation at the basal end is indicated by dotting. Fig. 5 is a case of new lateral polarity in a piece some twenty-five mm. in length from the middle of the naked region of a 70–80 mm. animal at a stage four days after operation. The apical hydranth of the piece is the second one developed, the first having been removed two days after section. The apical and the lateral hydranth are so near together that they mutually inhibit tentacle development on the sides facing each other and so have acquired a dorsoventrality with respect to each other. The side of the stem opposite the lateral hydranth shows an outgrowth which later becomes a base.



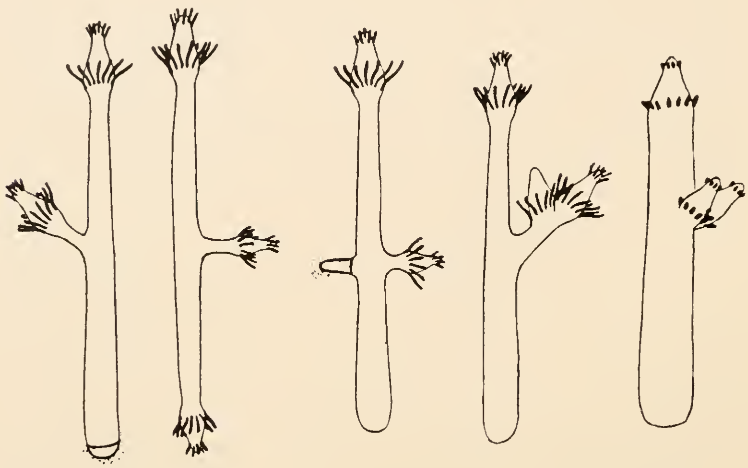
FIGS. 5–8. Development of new axes from lateral regions of growth determined by injury. Pieces 25 mm. in length from middle of naked region of animals 70–80 mm. Figures are about twice natural size. Figs. 5 and 6, two stages of a piece developing a complete new axis at right angles to the original polarity. Figs. 7 and 8, two stages of a piece which develops a new basal end in relation to both lateral and apical hydranth.

Fig. 6 represents the condition of the piece three days later. The two hydranths have now developed separate stems and a new base has arisen opposite the lateral hydranth. This development of a new individual from the apical end basipetally following the localization of the new apical end by the injury is an excellent example of apicobasal dominance. The localization of an active region by the injury determined a new hydranth, this determined successive stem regions basipetally until finally a new basal end was determined on the opposite side of the piece, that is, the dominance of the new axis became effective through the stem at right angles to the original polarity. Contact with the substratum may have assisted in determining the lateral stem region as a basal end (Child, '27*a, b, c*), though this region was not found in contact when the piece was observed, but if the new polarity were not concerned in localizing the base it would probably have arisen at or near the proximal end of the piece. At this stage then the piece represents two distinct polarities at right angles to each other. In later stages the form became bipolar-unipolar, the new base becoming the base for both hydranths and the stem region proximal to the lateral polarity gradually undergoing resorption.

Fig. 7 shows another case from the same series at a stage four days after operation. Here also the first apical hydranth was removed and the figure shows the second developing. Fig. 8 shows the same form three days later. Lateral stem regions are developing into a new basal end in relation to each hydranth. In this case as in the preceding, contact may perhaps have been concerned in producing conditions favorable to base development from the side of the piece, but the localization of the two bases in relation to the hydranths indicates that the more distal regions of each axis were to some extent concerned in localizing the bases. In this case also the region of the stem proximal to the lateral hydranth was gradually resorbed and the form became biapical and bibasal. The two individuals would probably have separated completely like most other double forms if they had been kept long enough.

In both of these cases the proximal stem region apparently cannot maintain itself in the presence of the new lateral polarity and is resorbed. The new polarity obliterates the old, probably because the new represents higher levels of metabolism and so is able to grow at the expense of the older stem regions. Such growth of new axes at the expense of old stem regions has been observed frequently in other experiments (Child, '27*a*).

Figs. 9–13 show cases from another series in which the pieces included the whole or almost the whole length of the naked region of 50–60 mm. animals. The figures show stages five days after section. In Figs. 9 and 10 the lateral axes have not developed basal ends, but in Fig. 11 a basal end is developing opposite the lateral hydranth and in Fig. 12 two hydranths have developed from the lateral outgrowth. Fig. 13, a piece of another series from 70–80 mm. animals three days after section is another case of two lateral hydranths.

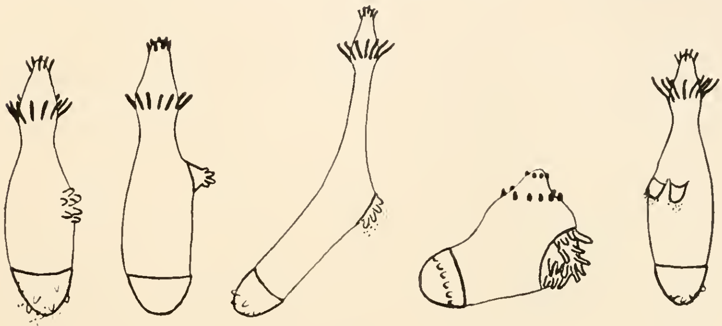


9 10 11 12 13

FIGS 9–13. Other cases of lateral hydranth development from region of growth determined by injury. Figs. 9–12 from pieces including the whole naked region of 50–60 mm. animals. Fig. 13 from piece including whole naked region of 70–80 mm. animal. The figures are slightly above natural size.

This lateral operation has been performed on thirty-five stem pieces and of these fifteen, or forty-three per cent. have given rise under standard conditions to new lateral axes consisting of at least a hydranth and more or less stem. Twenty pieces, fifty-seven per cent., healed without giving rise to new axes. Among the new lateral axes three, *i.e.*, nine per cent. of the total, became complete by the development of a basal end from the lateral stem region opposite the lateral hydranth.

It has been shown in earlier papers (Child, '26*b*, '27*a*, *b*), that either cut end or both, or any other region of a piece may develop as a basal end under inhibiting external conditions. In the light of these results it is to be expected that regions of lateral injury may also be made to develop as basal structures under inhibiting conditions. Up to the present, however, only one experiment of this sort under inhibiting conditions has been carried out, but its results are conclusive. Of the twenty pieces each including the naked and half the perisarcal region, which were used in this experiment fourteen, seventy per cent. gave rise to basal structures in the region of lateral injury and one, five per cent., to a hydranth,



14 *15* *16* *17* *18*

Figs. 14–18. Cases of development under inhibiting conditions of basal structures from lateral growing region determined by injury. Pieces include whole naked and half perisarcal region of 50 mm. animals. Figures are about twice natural size.

while five, twenty-five per cent., healed without outgrowth. Figs. 14–18 show characteristic cases from this series four days after operation. In Fig. 14 the region of injury has developed merely a few holdfast or stolon buds, while in Figs. 15 and 16 stolon buds are present at the tip of a general basal outgrowth. In the case of Fig. 17 the stolon buds in the region of injury are more numerous and attain greater length than in any other case. In Fig. 18 the region of injury has given rise to two basal outgrowths. These cases are sufficient to demonstrate that the lateral injury may develop as a basal as well as an apical end.

Discussion.

The experiments described above show that it is possible to localize new polarities in *Corymorpha* stems even without previously obliterating or decreasing the original polarity by means of inhibiting agents. It is important to note that these new polarities resulting from lateral injury are not similar to the lateral partial discs which result from a transverse cut part way through the body in *Cerianthus* (Loeb, '91, Child, '05, '08) and *Harenactis* (Child, '09*a*). In those forms the opening remains because the cut edges of body wall and oesophagus unite and the new partial disc develops entirely on the proximal side of the cut, just as a disc develops on any distal cut end of a piece. In the case of *Corymorpha* the wound closes completely in the course of a few hours and it is only through the continued growth of the region after closure of the wound that the new axis is determined. If the injury does not initiate such growth no new axis develops.

These new lateral polarities are essentially induced buds and like other buds they give us important evidence concerning the origin and nature of new axes. If we observe, without theoretical prejudice, what happens in such a process, we see that the new axis originates as a local region of growth and becomes visible as an outgrowth of the body wall (Fig. 2) because the growth activity is evidently greatest in its middle region and decreases peripherally in all directions. The early rounded outgrowth undergoes elongation and the more active middle region necessarily becomes its tip (Fig. 3), in other words, the region of growth has now become a physiological axis characterized by a gradient in activity decreasing from the tip basipetally. There can be no doubt that when such a gradient is once determined in a particular kind of protoplasm the constitution of the protoplasm will play the chief part in determining its steepness, its length and the changes which it undergoes during development. If we admit this, it follows that however the gradient is determined its definitive form will be the same in a protoplasm of a certain constitution, consequently a gradient such as the one under consideration, determined by a local injury will determine the same course of development, *i.e.*, the same kind of an axis as the gradient in embryonic development, if the condition of the cells in the region of active growth is similar to that of embryonic cells of *Cormorpha*. If we take the facts as they stand it seems that there is no adequate reason to regard a polar axis in its simplest form as anything more than such a gradient as this. Experiment shows that when such a gradient is determined a new axis is determined and when the gradient is obliterated the polarity is obliterated, so far as can be determined.

It has been shown for many forms, both plant and animal, that buds originate as gradients of this sort, resulting merely from the localization of an active region which is not sharply marked off from its surroundings but shows a gradient of decreasing activity from a central region toward the periphery. In consequence of differential growth, which itself results from the existence of this gradient, the radial gradient becomes an apicobasal gradient and a polar axis. There is no evidence to indicate that a polar axis is primarily anything more than such a dynamic differential with its structural protoplasmic correlates, or that differentiation along an axis requires anything more for its initiation than the quantitative differences at different levels of such a gradient.

The development of basal instead of apical structures from a lateral injury under inhibiting conditions is in complete agreement with the results of other experiments. It has been shown that the basal region of *Corymorpha* represents a secondary gradient which originates at the low end of the primary gradient (Child and Hyman, '26; Child, '26*a*). The high end of this secondary gradient, so long as it persists, is the basal tip and the slender modified stolons which constitute the holdfasts develop as lateral buds along this secondary gradient. These stolons show extremely rapid growth, but they originate only in regions of relatively low activity. When the activity of the region of lateral injury is decreased to a certain degree by inhibiting factors, the conditions must become more or less similar to those existing at the lower end of the primary gradient, and the lateral injury, like the lower end of the primary gradient, develops as a basal region. Whether a single basal outgrowth bearing stolon buds, or merely the stolon buds appear probably depends on various factors, *e.g.,* the degree of inhibition, the presence or absence of a definite growth region, etc. If a single general basal outgrowth arises the further development of the basal gradient and basal region follows in the same way as the development of the hydranth-stem gradient and region. Even if the central growth area resulting from the injury is not sufficiently well defined to determine a single general basal outgrowth, new stolon buds may be determined in relation to the entodermal canals or parts of canals in the injured region. Since the canals have been mutilated by the injury the arrangement of the stolon buds is likely to be irregular, as in Figs. 14–18.

Development of two apical or basal ends from the region of injury is undoubtedly a result of determination by the injury of two regions of activity instead of one. Duplication of this kind has been very widely observed in many forms as a result of splitting or otherwise dividing a growing region into two.

One of the most interesting results of these experiments is the determination by the more distal levels of the new axis of a basal region on the opposite side of the stem where there is no injury (Figs. 5, 6, 8, 12). It is evident that the development of the distal region of the new axis has in some way altered conditions in the region of the stem which gives rise to the base, but it has been shown that contact or nearness to the bottom and the action of various inhibiting agents may alter conditions in the same direction in regions of the stem (Child, '26*b*, '27*a*, *b*). This being the case there is no good reason for supposing that the changes which initiate the development of the basal end are anything more than quantitative changes in physiological condition determined by the presence of the new gradient. In the case of Fig. 8 in which the apical hydranth takes up a more or less lateral position because of the position of the piece, it, as well as the lateral hydranth, develops a new basal end, perhaps with the assistance of the conditions resulting from contact of the region concerned with the bottom.

The new lateral axis develops the characteristic radial symmetry, except in cases such as Fig. 5, in which the differential resulting from proximity of the other hydranth determines mutual and opposed dorsoventrality. If we examine the facts, again without theoretical prejudice, it appears that the radial symmetry of the axis is primarily nothing more than likeness of all radii in a plane perpendicular to the polar axis. The primary growing region determined by the injury is more or less radially symmetrical because its activity decreases radially from a center and as it becomes a definite outgrowth (Figs. 2, 3) its radial symmetry appears to result from this radial differential and from the fact that a surface-interior differential exists at all points. With the localization of tentacles certain radii become different from others. The factors concerned in tentacle localization have been but little investigated, but it is difficult to believe that the localization begins independently of external factors of some sort which determine where the first tentacle or tentacles shall appear. In the ordinary course of reconstitution the entodermal canals are important factors in localizing the new tentacles, as Torrey has shown (Torrey, '10). Factors concerned in tentacle localization in the lateral polarities have not been studied, but they will probably be found in the relations of the growing region to the rest of the stem. Study of *Hydra* and various hydroids indicates that localization of a single tentacle is sufficient to initiate the orderly development of others. Torrey's study of the order of appearance of tentacles in the embryonic development of *Corymorpha* is interesting in this connection as indicating that different localizing factors are concerned in different individuals for he finds that the process does not follow a uniform course (Torrey, '07). Apparently each region of growth, whether tentacle or other organ, dominates a certain area so that a similar organ cannot develop within that area. When a particular tentacle is localized, for example, another can develop only outside its range of dominance. Any part of the circumference in the tentacle forming region is undoubtedly capable of giving rise to a tentacle, but the actual localization in a particular case must depend on the factors concerned. That the outgrowth which becomes a polar axis with a radial symmetry can localize its own tentacles independently of any of the external differentials to which it is exposed is at least highly improbable and seems to require the action of some non-mechanistic ordering factor. If these observations and suggestions are correct, radial symmetry in these new axes has its origin in the primary likeness of radii at any particular level and in the difference between surface and interior which is present in some form in all organisms. The later localization of a series of similar organs in radial arrangement seems to demand the action of some differential external to the parts concerned.

Summary.

1. Buds have never been seen to arise from lateral stem regions in *Corymorpha* and a simple transverse cut into the side of the stem closes rapidly without development of a bud or other outgrowth, unless it extends almost through the stem.

2. A region of injury produced on the side of the stem by short cuts radiating from a center closes less rapidly than the simple transverse cut and in many cases gives rise after closure to a rounded outgrowth which becomes conical and develops into a new hydranth. This hydranth develops a stem at the expense of the old stem and in some cases the new axis determines a new basal end on the opposite side of the piece, thus completing a polarity at right angles to the original axis. Occasionally two hydranths instead of one are localized by the injury.

3. The experimental data indicate that the new polarity and symmetry are the necessary consequence of the localization of a center of cellular activity. The radial gradient of decreasing activity from the center peripherally becomes, as growth proceeds, the axial gradient and the radial symmetry is primarily merely a similarity of all radii vertical to the polar axis at any level.

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